

**Cytogenetic Studies on Wild *Chrysanthemum sensu lato* in China¹⁾. I.
Karyotype of *Dendranthema vestitum***

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中国産野生キク属（広義）の細胞遺伝学的研究¹⁾. I.

Dendranthema vestitum（ウラゲノギク）の核型

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The chromosome number of $2n = 54$ and the meiotic chromosome configuration of 27II at diakinesis and metaphase I in *Dendranthema vestitum* (Hemsl.) Ling, an endemic species of China, are reported here for the first time. This species is similar to *D. occidentali-japonense* (Nakai) Kitam. (= *Chrysanthemum japonense* Nakai) of Japan with respect to chromosome number ($2n = 54 = 6x$) and morphology of flower-head, but the karyotypes of the two species are different from each other. On the other hand, *D. vestitum* is relatively similar to the diploid ($2n = 18$) *D. japonicum* (Makino) Kitam. (= *C. makinoi* Matsum. et Nakai) of Japan with the common characters of the white-colored ligule of the flower-head, the simple-shaped leaf, morphology of the sat-chromosome, and presence of sub-terminal centromeric chromosomes. Since the chromosome complement of the hexaploid *D. vestitum* is almost equivalent to three times as that of the diploid *D. japonicum*, these two species might be closely related to each other.

Dendranthema vestitum (Hemsl.) Ling (Fig. 1) is restricted to Henan, Hubei and Anhui Provinces, China (Shih and Fu 1983). This species is paid much attention to be studied in cytology because it has some resemblance to a Japanese diploid ($2n = 18$) species, *D. japonicum* (Makino) Kitam. (= *Chrysanthemum makinoi* Matsum. et Nakai),

in having white ligules and simple-shaped leaves. It seems valuable to compare karyotypes between these two species when we detect and speculate evolutionary history of *Dendranthema* in East Asia.

We reveal and report here the chromosome number of $2n = 54$ (hexaploid) and the karyotype

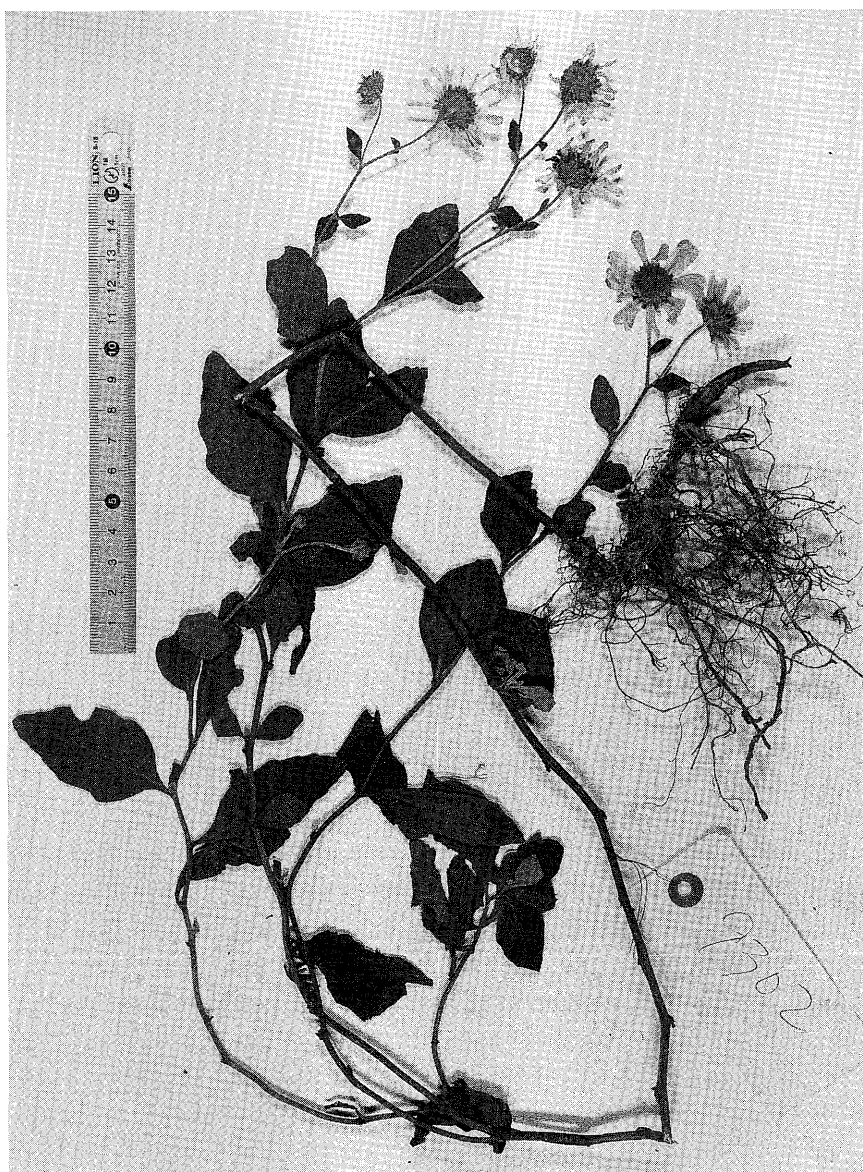


Fig. 1. A chromosome voucher ($2n = 54$, Nakata 9302) of *Dendranthema vestitum* collected in Huanghuachang, near Yichang City, Hubei Province, China.

of *D. vestitum* in comparisons with those of Japanese *Dendranthema* species.

Materials and Methods

During the second field survey in China in 1989 for the Monbuscho International Research Program-Joint Research, Nakata, Qiu and Uchiyama collected 30 living individual-plants of

Dendranthema vestitum in two populations near Yichang City, Hubei Province; 18 plants in Jiangjiawan, 10km northeast of Yichang, 230 m in altitude, and 12 plants in Huanghuachang, 20 km northeast of Yichang, 190 m in altitude. The former population was found on a roadside-slope along with an open pine forest associated with *Quercus*, *Vitex*, *Artemisia*, *Senecio*, *Xanthium*,

Kalimeris, *Patrinia* and so on. The latter population was seen on another roadside-slope with same but more sunny environment than the former one, with association of *Senecio*, *Bidens*, *Miscanthus*, *Eupatorium* and so on. Thirteen individual-plants of *D. vestitum* were introduced to Japan for the first time and cultivated in the experimental greenhouse of Hiroshima University for cytological studies.

Freshly growing root tips (ca. 1 cm long) were obtained from the cultivated plants and pretreated in 8 mM 8-hydroxyquinoline at 20°C for 3 hr. They were fixed in Farmer's fixative (3:1 mixture of ethanol and glacial acetic acid) at 5°C for 20 hr before they were macerated in 2:1 mixture of 45% acetic acid and 1 N HCl at 60°C for 10 sec and stained with 1% aceto-orcein for 10 min. Then, the meristematic tissues (ca. 1 mm long) of the root tips were squashed with 1% aceto-orcein for chromosome observation.

For karyotype study, the enzymatic maceration-flame drying method was also applied. Fixed root tips were transferred to deionized water through decreasing ethanol series (70, 50, 30, and 15%). Then, their meristematic tissues (ca. 1 mm long) were cut and placed onto slides and added one drop of enzyme mixture (4% cellulase "ONOZUKA" RS and 2% pectolyase Y-23, pH 4.8). The slides were incubated at 37°C for 40 min in a moistened petri dish. The root tips were rinsed with deionized water and re-fixed with Farmer's fixative before flame-drying. The chromosomes were stained with 1% aceto-orcein for 5 min, rinsed with tap water, and then air-dried.

Young flower heads were fixed in modified Carnoy's fixative (2:1:1 mixture of ethanol, chloroform and glacial acetic acid) and their anthers were squashed in 1% aceto-orcein. Meiotic chromosomes were observed at diakinesis and metaphase I in pollen mother cells (PMC's).

Measurements of chromosome lengths were made on photographs magnified $\times 3,000$ by a digitizer-personal computer system (Uchiyama et al. 1988). Nomenclature for centromeric positions of the chromosomes followed Levan et al. (1964).

The voucher specimens were deposited in the herbarium, Institute of Botany, Academia Sinica (PE).

Results and Discussion

Dendranthema vestitum showed morphological variation: the individual plants of the Jiangjiawan population displayed that flower heads varied from 27 to 47 mm in diameter. They also exhibited variations of lengths (7.5–17.5 mm), widths (3.5–5.5 mm) and number (13–21) of ligulate flowers, and of shapes (elliptic, oblong-ovate, ovate or rhombate) and bases (acute, obtuse, attenuate or truncate) of leaves.

At mitotic metaphase, $2n=54$ chromosomes (Fig. 2A) were observed in the 18 individuals of *D. vestitum* collected in the two populations (Accession numbers: Nakata 9261, 9265, 9268, 9269, 9270, 9271, 9272, 9273, 9278, 9279, 9280 and 9281 from the Jiangjiawan population; and 9291, 9297, 9298, 9299, 9300 and 9302 from the Huanghuachang population). At diakinesis (Fig. 2B) and meiotic metaphase I, $2n=27$ II chromosomes were also observed. Thus, the above is the first report of the chromosome number of *D. vestitum*. Since the basic chromosome number of *Chrysanthemum sensu lato* is $x=9$ (Shimotomai 1933), *D. vestitum* is a hexaploid ($2n=54=6x$) species.

Figure 2C shows a set of the individual chromosomes of *D. vestitum* arranged in decreasing order of the length. Thus, the $2n=54$ chromosomes varies gradually in length from 6.9 to 4.3 μm . Six medium- to small-sized subterminal centromeric (st) chromosomes (Fig. 2C) were easily identified



Fig. 2. Chromosomes of *Dendranthema vestitum*. A. Mitotic metaphase chromosomes in root tip cells of a plant from the Jiangjiawan population (Nakata 9278). B. Meiotic chromosomes at diakinesis ($2n=27II=54$) in PMC's of a plant (Nakata 9265) from the Jiangjiawan population. C. Individual chromosomes of a plant (Nakata 9265) prepared by enzymatic maceration-flame drying method. Asterisks show subterminal chromosomes. Stars show submedian chromosomes with a minute satellite. All bars indicate $5\ \mu\text{m}$.

from the others. Satellites often occurred on the short arms of the "st" chromosomes and/or small-sized submedian centromeric chromosomes (Fig. 2C).

With respect to flower head with white-colored ligules and the chromosome number of $2n=54$, *D. vestitum* of China and *D. occidentali-japonense* (Nakai) Kitam. (= *Chrysanthemum japonense* Nakai) of Japan are closely related to each other. However, their leaf shapes are quite different from each other; the leaf of the former species is not lobed, while that of the latter species is cleft or parted. Moreover, the karyotypes of the two species are different from each other; Watanabe

(1981) described that the karyotype of *D. occidentali-japonense* (as *C. japonense*) had eight or ten subterminal centromeric chromosomes and four median centromeric chromosomes with minute satellites.

Dendranthema vestitum is morphologically similar to *D. japonicum*, a diploid ($2n=18$) species of Japan, but their chromosome numbers are quite different from each other. According to Tanaka (1959) and Nakata (unpublished data), *D. japonicum* has two pairs of diagnostic chromosomes often exhibiting satellites; a pair of subterminal centromeric chromosomes (medium to small in size) and a pair of submedian to sub-

terminal centromeric chromosomes (small in size). Thus, *D. vestitum* and *D. japonicum* have the common feature in karyotype. Tripling of the diploid chromosome complement of *D. japonicum* might simulate the hexaploid karyotype of *D. vestitum*. *Dendranthema vestitum* seems to be karyomorphologically a close species to *D. japonicum* concerning a polyploid relation.

Dendranthema japonicum is distributed in the southwestern part of Japan except for Kyushu and the westernmost-part of Honshu (Nakata and Tanaka 1989). In contrast, *D. vestitum* grows on foothills or low mountains (340–1,500m in altitude) in Henan, Hubei and Anhui Provinces in China (Shih and Fu 1983). The distribution of *D. vestitum* seems to correspond to the transitional subtropics from the warm-temperate regions (Hou 1983), while that of *D. japonicum* correspond to the climatic regions of warm temperate to subtropics in Japan. The similarity of these distributions may also be correlated with their phylogenetic relationship.

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Endnote

1) A new series attached to the previous series entitled "Cytogenetic studies on wild *Chrysanthemum* from China (I–V)" (Tanaka and Taniguchi 1987, Taniguchi and Tanaka 1987, Tanaka et al. 1987a, 1987b, 1990), since we follow here Shih and Fu's taxonomic treatment (1983) for the Chinese genera *Dendranthema*, *Ajania*, *Tanacetum* and *Leucanthemella* and Kitamura's new treatment

(1978, 1981) for the Japanese genera *Dendranthema*, *Tanacetum*, *Leucanthemella* and *Nipponanthemum*, all segregated from *Chrysanthemum* sensu lato for convenience. The referable names under *Chrysanthemum* sensu lato used in our previous series (cf. Nakata et al. 1987) are also given in parentheses here.

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References

- Hou H.Y. 1983. Vegetation of China with reference to its geographical distribution. *Ann. Missouri Bot. Gard.* **70**: 509–548.
- Kitamura S. 1978. *Dendranthema* et *Nipponanthemum*. *Acta Phytotax. Geobot.* **29**: 165–170.
- 1981. *Compositae*. In: Satake Y. et al. (ed.), *Wild Flowers of Japan III*. Heibonsha, Tokyo (in Japanese).
- Levan A., Fredga K. and Sandberg A. A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* **52**: 201–220.
- Nakata M., Tanaka R., Taniguchi K. and Shimotomai N. 1987. Species of wild *Chrysanthemums* in Japan: Cytological and cytogenetical view on its entity. *Acta Phytotax. Geobot.* **38**: 241–259 (in Japanese).
- and Tanaka R. 1989. Species of *Chrysanthemum* in Japan in the study of chromosomes. In Hong D.Y. (ed.), *Plant Chromosome Research 1987*. p. 17–22. Organizing Committee of the Sino-Japanese Symposium on Plant Chromosomes, Beijing and Hiroshima.
- Shih C. and Fu G. X. 1983. *Compositae* (3), *Anthemideae*. *Flora Reipublicae Popularis Sinicae* **76**(1). Science Press, Beijing (in

Chinese).

- Shimotomai N. 1933. Zur Karyogenetik der Gattung *Chrysanthemum*. J. Sci. Hiroshima Univ. Ser. B, Div. 2, **2**: 1–100.
- Tanaka R. 1959. On the speciation and karyotypes in diploid and tetraploid species of *Chrysanthemum* II. Karyotypes in *Chrysanthemum Makinoi* ($2n=18$). J. Sci. Hiroshima Univ. Ser. B, Div. 2, **9**: 17–31.
- and Taniguchi K. 1987. Cytogenetic studies on wild *Chrysanthemum* from China I. Karyotype of *Ch. lavandulaefolium* var. *sianense*. CIS **42**: 10–12.
- , ——— and Aoyama M. 1987a. ditto. III. Karyotype of *Ch. arisanense*. CIS **43**: 20–21.
- , ———, Nakata M. and Aoyama M. 1987b. ditto. IV. Karyotype of *Ch. morii*. CIS **43**: 21–22.
- , Kawasaki S., Yonezawa Y., Taniguchi K. and Ikeda H. 1990. ditto. V. F_1 -hybrids of *Chrysanthemum lavandulaefolium* var. *sianense* \times *Ch. boreale*. Cytologia **54**: 365–372.
- Taniguchi K. and Tanaka R. 1987. ditto. II. Karyotype of *Ch. lavandulaefolium*. CIS **43**: 18–19.
- Uchiyama H., Nakata M., Morihito S. and Tanaka R. 1988. Use of personal computer and digitizer for karyotype analysis. La Kromosomo II-50: 1652–1658 (in Japanese).
- Watanabe K. 1981. Studies on the control of diploid-like meiosis in polyploid taxa of *Chrysanthemum* I. Hexaploid *Ch. japonense* Nakai. Cytologia **46**: 459–498.

要 旨

Dendranthema vestitum (Hemsl.) Ling (ウラゲノギク) は白色舌状花をもつ中国固有のキク属植物である。湖北省宜昌市近くの2集団で30個体の *D. vestitum* を採集し、観察した18個体の全てにおいて体細胞染色体数 $2n=54$ を算定した。また、花粉母細胞の減数分裂移動期、中期 I において、27個の二価染色体を観察した。キク属(広義)の染色体基本数は $x=9$ であるので、*D. vestitum* は6倍体である。*D. vestitum* の染色体組には、他の染色体と容易に区別できる中型ないし小型の次端部動原体型染色体が6個あり、付随体はこの次端部動原体型染色体と小型の次中部動原体型染色体にしばしば観察された。*D. vestitum* は白色舌状花を持ち、6倍体である点で日本産ノジギク *D. occidentali-japonense* (Nakai) Kitam. (= *Chrysanthemum japonense* Nakai) と似ているが、葉の形態と核型は異なっていた。一方、白色舌状花を持ち、葉の形が単純な点で、*D. vestitum* は日本産リュウノウギク *D. japonicum* (Makino) Kitam. (= *C. makinoi* Matsum. et Nakai) にも似ている。リュウノウギクは2倍体 ($2n=18$) であるが、次端部動原体型染色体が2個観察され、付随体染色体の形態も共通しており (Tanaka 1959)、リュウノウギクの染色体組を3倍すると、6倍体の *D. vestitum* に似た染色体組になる。核型的に、*D. vestitum* はリュウノウギクと倍数性の関係を持つ近縁種であることが示唆される。